Trends
in Psychiatry and Psychotherapy

Original Article

Evaluation of BDNF levels in patients hospitalized for physical trauma at an emergency hospital in Porto Alegre, southern Brazil

Avaliação dos níveis de BDNF em pacientes hospitalizados por trauma físico em um hospital de emergência de Porto Alegre, sul do Brasil

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Abstract

Objective: To assess the association between brain-derived neurotrophic factor (BDNF) levels and acute stress disorder (ASD) in patients who have suffered physical trauma.
Methods: Data were collected at an emergency hospital in Porto Alegre, state of Rio Grande do Sul, southern Brazil. Participants were over 18 years of age, victims of physical trauma, and had been hospitalized for a minimum of 48 hours. A total of 117 hospitalized patients who agreed to participate in the research were grouped according to the shift in which blood was collected (38 subjects from the morning shift and 79 from the afternoon shift), had their BDNF levels measured and responded to other questionnaires. Respondents were further grouped by age into three ranges: 18-30, 31-50 and 51-70 years.
Results: We found a significant difference in the distribution of BDNF between the two shifts in which blood samples were collected, with the afternoon group having higher BDNF levels (U = 1906.5, p = 0.018). A difference was observed only between the 18-30 group and the 51-70 group in the afternoon shift ($U_{morning}$ = 1107, $p_{morning}$ = 0.575; $U_{afternoon}$ = 7175, $p_{afternoon}$ = 0.028).
Conclusions: The population whose blood samples were collected in the afternoon showed significantly higher values of BDNF compared to those of the morning shift. This same population presented lower BDNF levels when associated with ASD subtypes A1, A2, and A. We hypothesize that the lower values of BDNF measured in the morning shift were due to a response to the circadian cycle of cortisol, whose action inhibits the expression of serum neurotrophins.
Keywords: Acute stress disorder, BDNF, physical trauma, trauma in childhood.

Resumo

Objetivo: Verificar a associação entre os níveis de fator neurotrófico derivado do cérebro (brain-derived neurotrophic factor [BDNF]) e transtorno de estresse agudo (TEA) em pacientes que sofreram trauma físico.
Métodos: Os dados foram coletados em um hospital de emergência de Porto Alegre, Rio Grande do Sul, Brasil. Os participantes eram maiores de 18 anos, vítimas de trauma físico e estavam hospitalizados por um período mínimo de 48 horas. Um total de 117 pacientes hospitalizados que concordaram em participar da pesquisa foram agrupados de acordo com o turno de realização da coleta de sangue (38 sujeitos no turno da manhã e 79 sujeitos no turno da tarde), tiveram seus níveis de BDNF medidos e responderam a outros questionários. Os entrevistados também foram agrupados por idade em três faixas etárias: 18-30, 31-50 e 51-70 anos.
Resultados: Encontramos uma diferença significativa na distribuição de BDNF entre os turnos, sendo que o grupo da tarde apresentou níveis maiores de BDNF (U = 1906,5, p = 0,018). Houve diferença entre o grupo de 18-30 anos e o de 51-70 anos no turno da tarde ($U_{manhã}$ = 1107, $p_{manhã}$ = 0,575; U_{tarde} = 7175, p_{tarde} = 0,028).
Conclusões: A população cuja coleta ocorreu à tarde apresentou valores significativamente maiores de BDNF em relação à coleta do turno da manhã. Esta mesma população apresentou menores níveis dessa neurotrofina quando associada com os subtipos A1, A2 e A de TEA. É possível hipotetizar que os menores valores de BDNF aferidos na coleta do turno da manhã se devam a uma resposta ao ciclo circadiano do cortisol, cuja ação inibe a expressão de neurotrofinas séricas.
Descritores: Transtorno de estresse agudo, BDNF, trauma físico, trauma na infância.

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Introduction

Acute stress disorder (ASD) is a diagnosis organized under disorders related to trauma and stressors introduced in the Diagnostic and Statistical Manual of Mental Disorders, 4th edition (DSM-IV). Since then, a series of works have attempted to evaluate to what extent ASD might be a predictor of posttraumatic stress disorder (PTSD).[1] However, the centrality defined by the DSM-IV says that symptoms need to last for only two days for a diagnosis of ASD to be made. Therefore, for the scope of the present study, the authors deemed it necessary to adopt a more up-to-date version of the DSM. Thus, for ASD only, we used DSM-5 criteria for diagnosis. In the DSM-5, ASD is characterized by the onset of a minimum set of symptoms 3 to 30 days after the person has been exposed to a traumatic event. Avoidance, peritraumatic dissociation, intrusive re-experiencing, hyperexcitation, and disruption of normal social/work functioning are the main symptoms listed. After the 30-day mark, diagnosis should be changed to PTSD.[2]

The National Comorbidity Survey Replication[3] estimates that approximately 6.8% of the general population will suffer from PTSD at some point in their lives, with the incidence being as high as 9.2% for individuals in the 45 to 60 age range. Women are more often affected than men, at a ratio of 2:1 when adjusted for the relative frequency of trauma between genders. There do not seem to be any studies on the prevalence of ASD in the general population, and prospective studies and systematic reviews differ according to the nature of the event and the context in which it is evaluated.[3]

Various research centers, primarily American, Israeli, and Australian, are noteworthy for undertaking and publishing multi-pronged investigative projects. They range from diagnostic validation, through the search for biomarkers and relevant neuroendocrine findings, to the proposal and testing of different therapeutic strategies for PTSD.[1,4]

Important public health resources may be expended in the post-diagnosis treatment of these individuals, as they often become incapacitated by chronic forms of PTSD or by depression and anxiety.[5,6] Add to it the prejudices brought on by being away from work and/or the loss of productivity due to treatments, and one can see the relevance of the problem.

In our area, studies on the presence of altered brain-derived neurotrophic factor (BDNF) levels in stress disorders have been appearing.[7,8] In light of the epidemiological evidence and the findings of the above-mentioned studies, the need to better understand ASD is clear. The role of BDNF in learning, motivation, memory, and mood regulation makes it an important protagonist in the investigation of neurobiological factors related to the posttrauma experience. Therefore, the present study sought to assess BDNF levels in patients who have suffered physical trauma and to investigate associations between BDNF levels and ASD subtypes, sociodemographic factors, and mental disorders according to blood collection shift.

Methods

Sample

Data were collected at an emergency hospital for physical trauma in Porto Alegre, state of Rio Grande do Sul, southern Brazil. Study participants were patients over 18 years of age, victims of sufficient physical trauma to require hospitalization, and for whom a minimum hospitalization time of 48 hours was recommended by the clinical physician. The studied population was divided according to the shift when blood was collected (morning or afternoon). ASD was diagnosed using DSM-5 criteria.[2]

Patients who refused to participate or who were not able to consent due to their clinical condition were excluded from the study. Patients hospitalized for suicide attempt were also excluded, as they were considered to require a high degree of care related to their psychopathology, which might interfere with this study's results.

The study used a convenience sample of 117 participants. Data collection occurred between July 22 2013 and December 19 2014.

Instruments

The Defensive Style Questionnaire (DSQ)

Defense mechanisms were evaluated via this instrument, developed by Bond et al.,[9] subsequently revised by Vaillant et al.,[10] and reorganized by Andrews et al.[11] as the DSQ-40. The same instrument was also used by Blaya et al., who evaluated defense mechanisms using a Portuguese version of the DSQ-40 and undertook a preliminary study of its adaptation and validation.[12]

Childhood Trauma Questionnaire (CTQ)

The CTQ was used to evaluate trauma and abuse in childhood. This tool can be applied to adolescents (12 years and older) and adults. The Brazilian Portuguese version of the instrument was reduced to 28 statements related to situations that occurred during childhood, rated using a 5-point Likert scale. Five dimensions of childhood trauma are investigated: physical abuse, emotional abuse, sexual abuse, physical neglect, and emotional neglect. Scores may range from 25 (absence

of trauma) to 125 (maximum score, indicating that all traumas are fully present).[13] The original questionnaire forms were bought in the United States to be applied in this study, with permission granted by Pearson Education for the use of assessment products of trauma in childhood.

Mini-International Neuropsychiatric Interview (MINI)

For the evaluation of psychiatric comorbidities, we used an interview compatible with the DSM-III-R/IV and the 10th edition of the International Statistical Classification of Diseases and Related Health Problems (ICD-10). Evaluation of ASD, however, was made using the five adapted DSM-5 criteria (labeled A through E). Briefly, patients diagnosed with ASD presented dissociative symptoms after exposure to trauma: a concrete episode or threat of death, serious injury, or sexual violence (criterion A). This criterion has four subtypes, A1, A2, A3, and A4, as follows: directly experienced a traumatic event (A1); witnessed, in person, a traumatic event happening to someone else (A2); learned of a traumatic event, either violent or accidental, that happened to a relative or close friend (A3); and experienced repeated or extreme exposure to adverse details of a traumatic event (A4). While hospitalized after the traumatic event, the individual had at least three of the following dissociative symptoms: subjective feeling of being anaesthetized, being distant, or lacking emotional response; reduced awareness of surroundings; altered sense of reality; depersonalization or dissociative amnesia (criterion B). During hospitalization, the event continued to be relived (criterion C). The subject presented marked aversion to stimuli that might activate memories of the trauma (criterion D). Finally, the individual had heightened symptoms of elevated anxiety or excitability or negative changes in mood and cognition (criterion E).[14] It is important to note that criterion A was subdivided to enable investigation of the specific types of trauma experienced by ASD patients.

Measurement (blood analysis)

Four milliliters of blood were drawn from each subject by venipuncture into an anticoagulant-free vacuum tube. The blood was centrifuged at 3000 g for 10 min and serum was kept frozen at -80°C until analysis. BDNF serum levels were measured by sandwich enzyme-linked immunosorbent assay (ELISA), using a commercial kit according to the manufacturer's instructions (Millipore, Billerica, MA, USA). Briefly, microtiter plates (96-well flat-bottom) were incubated for 24 hours at 4 °C with the samples diluted to 1:75 in sample diluent and a standard curve ranging from 15 to 1000 pg/ml of BDNF. Plates were then washed four times with wash buffer followed by the addition of biotinylated mouse anti-human BDNF monoclonal antibody (diluted to 1:1000 in sample diluent), which was incubated for 3 hours at room temperature. After washing, a second incubation with streptavidin-horseradish peroxidase conjugate solution (diluted to 1:1000) for 1 hour at room temperature was carried out. After addition of substrate and stop solutions, the amount of BDNF was determined (absorbance set at 450 nm). The standard curve demonstrated a direct relationship between optical density and BDNF concentration.

Statistical analysis

Categorical variables were described as relative and absolute frequency. Quantitative variables were presented as median and interquartile minimum and maximum, according to the results of the Shapiro-Wild normality test.

The chi-square test was used to compare the sociodemographic characteristics of the groups. The Mann-Whitney U or Kruskal-Wallis H tests were used for the independent samples, according to the type of variable and its distribution or due to a small sample size. Spearman's correlation was employed to assess the association between similar variables. The significance level adopted was 0.05. The analyses were performed in the Statistical Package for the Social Sciences (SPSS) version 18.

Ethical considerations

The present study is part of a broader research project entitled "Evaluation of acute stress disorder and posttraumatic stress disorder in patients hospitalized for physical trauma," approved by the research ethics committee of Hospital de Clínicas de Porto Alegre, Porto Alegre, state of Rio Grande do Sul (report #110533/2012). The study was also approved by the research ethics committee of the Municipal Health Department of the Municipality of Porto Alegre (CAAE 02910012.5.3001.5338), and is in accordance with all the ethical recommendations of Resolution 196/96 of the Brazilian National Health Council. The participants and/or their guardians were given all the necessary information, and any doubts related to the study were duly clarified. After this procedure, those who agreed to participate in the study signed an informed consent form. The anonymity of all patients is protected.

Results

A total of 117 patients admitted to the emergency hospital were evaluated. The reasons for their admission

were aggression or accident involving physical trauma. The subjects were grouped according to shift of blood collection: 38 subjects from the morning and 79 from the afternoon shift. Following BDNF measurement and questionnaire response analysis, one significant difference was found in the distribution of BDNF levels between the shifts: patients in the afternoon shift showed higher BDNF levels (U = 1906, p = 0.018) as presented in Table 1.

There were 103 male and 14 female participants. Intergender comparison did not reveal any significant differences ($U_{morning}$ = 74, $p_{morning}$ = 0.714; $U_{afternoon}$ = 358, $p_{afternoon}$ = 0.507). The majority had no previous psychiatric history ($U_{morning}$ = 33, $p_{morning}$ = 0.291; $U_{afternoon}$ = 605, $p_{afternoon}$ = 0.352). Participants were subdivided by age into three ranges: 18-30, 31-50 and 51-70 years old. A difference was observed only between the 18-30 group and the 51-70 group for the afternoon shift ($U_{morning}$ = 1107, $p_{morning}$ = 0.575; $U_{afternoon}$ = 7175, $p_{afternoon}$ = 0.028) (Table 2).

Table 1 - Differences in brain-derived neurotrophic factor levels according to shift of blood collection

Percent	Morning (n = 38)	Afternoon (n = 79)
Min	0.3	1.4
10	3.4	5.4
20	5.2	10.9
30	9.2	19.6
40	14.4	25.5
50	20.2	28.6
60	23.2	33.1
70	26.1	41.2
80	35.5	56.1
90	56.5	69.6
Max	89.2	132.2

Mann-Whitney test (p = 0.018).

The distribution of BDNF levels was compared between the ASD criterion and its subtypes. As seen in Table 3, only seven patients presented a positive diagnosis for general ASD (as opposed to one of its subtypes). Regardless of blood collection shift, no statistical differences were observed ($U_{morning}$ = 82, $p_{morning}$ = 0.983; $U_{afternoon}$ = 92, $p_{afternoon}$ = 0.640). Among the negative cases, the mean between the shifts differed by about 10 points. Also, a statistical difference was found between three subtypes of ASD, but only for the afternoon shift. Subjects with ASD subtypes A1 ($U_{afternoon}$ = 892, $p_{afternoon}$ = 0.013), A2 ($U_{afternoon}$ = 1005, $p_{afternoon}$ = 0.025), and A ($U_{afternoon}$ = 581, $p_{afternoon}$ = 0.044) presented a distribution of BDNF lower than that found in subjects without ASD.

Mental disorders were evaluated using the MINI instrument. The most prevalent disorders are presented in Table 4. When BDNF levels were compared according to shift, only substance abuse resulted significant in the afternoon shift ($U_{afternoon}$ = 218, $p_{afternoon}$ = 0.021).

The concentration of BDNF was examined in relation to age, number of ASD symptoms, DSQ domains, and scores

Table 2 - Description and comparison of the distribution of brain-derived neurotrophic factor levels according to sociodemographic factors and shift of blood collection

	Morning				Afternoon			
	Median [Q1; Q3]	Min-Max	n	p	Median [Q1; Q3]	Min-Max	n	p
Sex								
Male	20.6 [8.4; 28.8]	3.2-89.2	33	0.714	28.5 [13.5; 42.5]	1.8-132.2	70	0.507
Female	19.5 [18.3; 24.4]	0.3-26.5	5		38.3 [19.7; 49.9]	1.4-117.8	9	
Psychiatric history								
Yes	28.2 [14.9; 39.0]	14.9-39.0	3	0.291	27.0 [12.2; 38.2]	1.4-77.2	17	0.352
No	19.8 [6.5; 26.5]	0.3-89.2	35		30.7 [14.0; 46.4]	1.8-132.2	62	
Age range (years)								
18-30	16.6 [10.0; 27.3]	6.5-54.6	12	0.575	**20.1[a] [8.5; 33.1]**	**1.4-117.8**	**33**	**0.028**
31-50	23.3 [13.8; 27.3]	3.2-73.7	16		**30.2[ab] [19.7; 46.4]**	**1.9-132.2**	**33**	
51-70	10.1 [3.5; 34.6]	0.3-89.2	10		**38.6[b] [31.2; 52.5]**	**17.2-85.1**	**12**	
Admission reason								
Aggression	10.9 [6.5; 19.8]	0.3-28.8	9	0.135	25.6 [17.5; 41.7]	1.4-132.2	32	0.712
Accident	23.1 [8.8; 31.4]	3.2-89.2	24		30.7 [11.5; 50.9]	1.8-117.8	36	

Min-Max = minimum-maximum; Q = quartile.
Different superscript letters indicate statistical differences.

in the CTQ domains. Spearman's correlation was calculated to measure the degree to which these variables and BDNF levels were related. Significant correlations were found only for the afternoon shift. Age was the variable with the greatest correlation, showing a moderate degree ($r_{afternoon}$ = 0.34, p = 0.003). Among the defense mechanisms, rationalization ($r_{afternoon}$ = 0.22, p = 0.050) and reaction formation ($r_{afternoon}$ = 0.27, p = 0.015) presented significance (Table 5). These correlations were classified as weak. Trauma did not show significant results.

Table 3 - Comparison of the distribution of brain-derived neurotrophic factor levels according to types of ASD and shift of blood collection

	Morning				Afternoon			
	Mean [Q1; Q3]	**Min-Max**	**n**	**p**	**Mean [Q1; Q3]**	**Min-Max**	**n**	**p**
ASD A1								
Yes	21.3 [8.4; 26.5]	0.3-89.2	30	0.830	27.0 [10.9; 38.8]	1.4-117.8	55	**0.013**
No	16.6 [7.4; 46.8]	3.4-86.0	8		39.6 [23.8; 67.0]	3.1-132.2	24	
ASD A2								
Yes	20.0 [5.3; 24.5]	0.3-89.2	22	0.408	22.8 [8.5; 38.2]	1.4-132.2	37	**0.025**
No	21.2 [9.2; 33.9]	3.4-86.0	16		31.7 [21.6; 56.2]	3.1-79.4	42	
ASD A3								
Yes	20.0 [5.8; 27.6]	0.3-89.2	20	0.640	25.7 [9.1; 41.2]	1.8-85.1	35	0.236
No	21.0 [9.2; 28.2]	3.4-86.0	18		31.5 [18.7; 46.1]	1.4-132.2	44	
ASD A4								
Yes	22.0 [9.2; 28.2]	0.3-89.2	15	0.800	28.9 [8.9; 45.7]	1.9-85.1	19	0.783
No	19.5 [6.5; 28.8]	3.2-86.0	23		28.5 [13.7; 45.0]	1.4-132.2	60	
ASD A								
Yes	19.8 [8.4; 25.9]	0.3-89.2	33	0.235	27.2 [13.0; 41.8]	1.4-132.2	66	**0.044**
No	39.0 [10.9; 54.6]	3.8-86.0	5		41.0 [27.1; 66.5]	3.1-79.4	13	
ASD B								
Yes	21.7 [8.7; 25.4]	0.3-34.6	8	0.802	19.9 [8.5; 41.2]	1.4-51.9	10	0.288
No	20.2 [8.4; 28.8]	3.2-89.2	30		28.9 [17.7; 46.4]	1.8-132.2	69	
ASD C								
Yes	20.2 [8.4; 28.2]	0.3-89.2	38	-	28.6 [13.5; 45.7]	1.4-132.2	79	-
No			0				0	
ASD D								
Yes	24.2 [8.7; 30.5]	0.3-89.2	16	0.460	27.5 [13.5; 49.9]	4.1-132.2	27	0.975
No	19.1 [8.4; 28.2]	3.2-86.0	22		29.6 [13.5; 43.0]	1.4-117.8	52	
ASD E								
Yes	25.4 [19.5; 28.8]	5.3-89.2	10	0.079	26.6 [9.1; 41.0]	1.8-77.2	18	0.520
No	16.6 [5.8; 25.2]	0.3-86.0	28		30.2 [14.0; 46.4]	1.4-132.2	61	
ASD								
Positive	24.0 [19.5; 24.4]	3.4-26.5	5	0.983	23.6 [5.9; 41.2]	5.9-41.2	2	0.640
Negative	19.8 [8.4; 28.8]	0.3-89.2	33		28.6 [14.0; 45.7]	1.4-132.2	77	

ASD = acute stress disorder; Min-Max = minimum-maximum; Q = quartile.
Mann-Whitney U test.
ASD subdivided by criteria (A, B, C, D, E).

Table 4 - Comparison of the distribution of brain-derived neurotrophic factor levels according to mental disorder and shift of blood collection

	Morning				Afternoon			
	Mean [Q1; Q3]	**Min-Max**	**n**	**p**	**Mean [Q1; Q3]**	**Min-Max**	**n**	**p**
Current depression								
No	21.3 [9.3; 34.6]	3.2-89.2	26	0.370	28.8 [17.2; 46.4]	1.4-132.2	58	0.807
Yes	14.9 [5.3; 24.5]	0.3-51.1	11		28.4 [13.0; 41.2]	4.1-73.6	21	
Current melancholy								
No	20.6 [9.3; 28.8]	3.2-89.2	29	0.461	27.3 [13.0; 43.0]	1.4-132.2	64	0.194
Yes	16.8 [5.8; 24.2]	0.3-51.1	8		38.0 [25.5; 51.9]	5.9-73.6	15	
Past depression								
No	19.8 [8.4; 28.2]	3.2-89.2	31	0.564	28.6 [17.2; 46.4]	1.4-132.2	63	0.634
Yes	25.2 [6.5; 51.1]	0.3-73.7	6		28.4 [8.9; 45.7]	4.1-69.3	15	
Suicide risk								
No	22.0 [9.2; 28.2]	0.3-89.2	19	0.902	26.7 [14.0; 41.8]	1.9-117.8	46	0.256
Yes	18.3 [9.3; 24.4]	3.5-86.0	9		33.1 [21.9; 51.9]	1.4-132.2	21	
Lifetime psychosis								
No	19.8 [6.5; 25.9]	3.2-86.0	27	0.584	31.2 [19.6; 49.9]	1.8-132.2	62	0.061
Yes	22.0 [13.7; 28.8]	0.3-51.1	10		21.9 [8.5; 31.4]	1.4-73.2	17	
Primary agoraphobia								
No	20.6 [5.3; 28.2]	0.3-89.2	29	0.356	28.8 [17.2; 45.7]	1.4-132.2	66	0.533
Yes	21.9 [14.3; 32.7]	9.3-73.7	8		24.3 [11.5; 39.6]	5.9-73.6	12	
Social anxiety disorder								
No	20.6 [9.2; 28.8]	3.2-89.2	29	0.400	29.6 [14.0; 46.4]	1.4-132.2	66	0.539
Yes	18.3 [6.5; 24.4]	0.3-34.6	9		23.5 [13.0; 41.2]	5.9-69.6	10	
Current alcoholism								
No	20.2 [9.2; 28.2]	0.3-89.2	30	0.832	31.1 [17.2; 43.5]	1.8-132.2	53	0.900
Yes	19.5 [6.5; 24.5]	3.8-34.6	6		27.2 [13.0; 51.9]	1.4-77.2	26	
Alcohol abuse								
No	20.2 [8.4; 28.2]	0.3-89.2	34	0.738	29.4 [13.7; 46.1]	1.4-132.2	60	0.905
Yes	14.9 [6.5; 24.5]	6.5-24.5	3		27.5 [13.0; 41.8]	5.9-77.2	15	
Substance dependency								
No	19.5 [5.3; 28.2]	0.3-89.2	31	0.801	28.6 [13.0; 45.7]	1.4-132.2	55	0.900
Yes	20.6 [9.2; 22.7]	6.5-24.0	5		27.0 [13.5; 51.9]	1.8-73.2	23	
Substance abuse								
No	19.7 [5.3; 28.2]	0.3-89.2	30	0.966	31.1 [19.7; 51.9]	1.4-117.8	63	**0.021**
Yes	15.7 [9.3; 25.9]	8.4-39.0	6		11.3 [8.4; 32.3]	1.9-45.7	12	
Current psychosis								
No	20.6 [8.4; 28.8]	3.2-89.2	31	0.638	30.7 [15.8; 48.2]	1.8-132.2	64	0.151
Yes	18.3 [3.8; 26.5]	0.3-34.6	7		25.7 [8.2; 33.1]	1.4-73.2	15	
Current mental disorder								
No	3.4 [3.2; 86.0]	3.2-86.0	3	0.401	38.3 [28.6; 43.5]	5.4-79.4	5	0.469
Yes	20.6 [9.2; 28.2]	0.3-89.2	35		27.9 [13.5; 45.7]	1.4-132.2	74	

Mann-Whitney U test.

Table 5 - Correlations between age, number of ASD symptoms, defense mechanisms, childhood trauma and brain-derived neurotrophic factor levels according to shift of blood collection

	Morning *r* (p)	Afternoon *r* (p)
Age	-0.11 (0.514)	**0.34 (0.003)**
ASD A		
Number of symptoms	-0.10 (0.568)	**-0.28 (0.011)**
Defensive Style Questionnaire		
Mature	0.15 (0.382)	0.19 (0.105)
Anticipation	0.09 (0.603)	0.11 (0.325)
Humor	0.22 (0.190)	0.01 (0.927)
Suppression	0.18 (0.266)	0.07 (0.533)
Sublimation	-0.05 (0.758)	0.17 (0.143)
Rationalization	0.00 (0.980)	0.22 (0.050)
Neurotic	0.14 (0.405)	0.18 (0.118)
Pseudo-altruism	0.12 (0.466)	0.03 (0.820)
Idealization	0.16 (0.336)	0.01 (0.926)
Reaction formation	0.16 (0.334)	0.27 (0.015)
Undoing	-0.06 (0.737)	0.15 (0.199)
Immature	-0.11 (0.521)	-0.02 (0.887)
Projection	-0.03 (0.848)	-0.06 (0.585)
Passive aggression	-0.21 (0.216)	-0.03 (0.772)
Acting out	-0.29 (0.082)	-0.11 (0.335)
Isolation	0.12 (0.472)	-0.14 (0.214)
Devaluation	-0.27 (0.107)	0.02 (0.847)
Autistic fantasy	-0.16 (0.343)	0.19 (0.102)
Denial	0.11 (0.528)	-0.06 (0.618)
Displacement	-0.07 (0.683)	-0.05 (0.690)
Dissociation	0.05 (0.744)	0.00 (0.975)
Splitting	-0.05 (0.766)	-0.02 (0.837)
Somatization	-0.02 (0.922)	0.03 (0.779)
Childhood Trauma Questionnaire		
Emotional abuse score	0.05 (0.794)	-0.15 (0.190)
Physical abuse score	0.23 (0.200)	-0.02 (0.884)
Sexual abuse score	-0.02 (0.926)	-0.15 (0.181)
Emotional neglect score	-0.12 (0.516)	0.09 (0.445)
Physical neglect score	0.21 (0.225)	-0.13 (0.271)
Total score CTQ	0.16 (0.381)	-0.15 (0.202)

ASD = acute stress disorder.
Spearman's correlation.

Discussion

BDNF is a neurotrophin involved in neuronal growth and survival as well as synaptic plasticity.[15] It is highly expressed in mammal brains, particularly in the hippocampus, whose function is associated with learning and memory.[8,16] In this way, BDNF participates in the formation of long-term memory and other cognitive processes and is an important factor to be evaluated in patients who have developed PTSD.[17]

Current studies indicate that factors such as genetics, childhood environment, previous trauma, and psychological and cognitive factors are related to the development of traumatic stress. As it plays a crucial role in neuronal processes, BDNF has been intensely studied in the last decade.[1,4,15]

In our literature search, no studies with similar findings were encountered, and we hypothesized that this could be due to the influence of circadian endogenous cortisol variations on BDNF serum production. Authors report that cortisol secretion intensity is higher in humans early in the morning and lower at the end of the day, varying from a maximum of approximately 20 µg/dl one hour before awakening in the morning to a minimum of about 5 µg/dl around midnight.[18] A recent study has revealed that glucocorticoid receptors down-regulate the expression of BDNF.[19] In that study, the authors demonstrated that exposure to dexamethasone suppressed the expression of the transcription of BDNF in neuronal cells mediated by glucocorticoid receptors. Thus, based on the hypothesis above, it can be inferred that greater endogenous cortisol serum levels may be associated with diminished BDNF serum expression, leading to the findings of our study (Table 1).

With this in mind, studying BDNF levels in patients who have suffered physical trauma, whether they developed ASD or not, has uncovered a significant difference in the distribution of BDNF levels in blood samples collected in morning vs. afternoon shifts, with higher BDNF levels found in the group tested in the afternoon ($U = 1906.5$, $p = 0.018$), as seen in Table 1. Studies show that lowered BDNF expression is implicated in greater sensitivity to stress and an increased response to that stress.[20,21] Previous results have shown that rats heterozygous for the gene that codes for BDNF were more vulnerable to stress than controls, exhibiting desperation behavior after the stress of mild handling. The BDNF genotype Met/Met has also been associated with a significant proportion of soldiers with traumatic stress. However, only 5.2% of the total sample presented the Met/Met genotype. In the literature, these findings, together with BDNF polymorphisms, have shown strong association with synaptic plasticity and mood disorders, which have also been associated with greater risk for PTSD.

The literature still indicates that patients with previous traumatic stress, with greater exposure to combat, with a more sustained mild traumatic brain injury during the trauma, and who carry the Met/Met BDNF genotype accounted for 22% of the variance in posttrauma PTSD scores ($r^2 = 0.22$, $p < 0.001$).[22] In our sample, no correlation was found between previous trauma, development of ASD and changes in BDNF levels.

In one study, low levels of BDNF serum were associated with PTSD, while in another, no relationship was observed with PTSD in victims of urban violence.[23] However, the Met allele of the BDNF Val66Met polymorphism might be associated with severity of PTSD in war veterans.[24]

Regardless of sample collection shift, no statistical differences were found between the ASD criterion and its subtypes ($U_{morning} = 82$, $p_{morning} = 0.983$; $U_{afternoon} = 92$, $p_{afternoon} = 0.640$). Among the negative cases, the mean between shifts showed a difference of almost 10 points, which was also observed in a study involving 16 unmedicated emergency patients diagnosed with chronic PTSD due to sexual and/or physical abuse or automobile accidents, in which the authors found similar BDNF serum concentrations for patients with PTSD and patients without the condition (controls) (1.00±0.52 vs. 0.83±0.44 pg/ml, $p = 0.39$).[25]

A statistical difference was found between three subtypes of ASD, only for the afternoon shift. Subjects with ASD A1 ($U_{afternoon} = 892$, $p_{afternoon} = 0.013$), A2 ($U_{afternoon} = 1005$, $p_{afternoon} = 0.025$), and A ($U_{afternoon} = 581$, $p_{afternoon} = 0.044$) presented a lower distribution of BDNF levels when compared to subjects without ASD, which corroborates another study conducted with 23 unmedicated emergency patients diagnosed with PTSD. In that study, diminished BDNF serum concentration was reported for the patients with PTSD as compared to the controls (4018.1±359 vs. 4886.6±180 pg/ml, $p < 0.05$).[26]

One study investigated the protective effects of BDNF against the damaging effects of oxidation induced by stress in rats. Rats deficient in BDNF were more susceptible to oxidative damage caused by stress, suggesting that a direct interaction exists between the indicators of oxidative stress and BDNF levels in the brain.[27]

Moreover, the results of the study indicated no significant differences in oxidative stress biomarkers between rats heterozygous for BDNF and wild-gene ones that did not suffer stress.

Parallel to these findings, it has been demonstrated that oxidative stress could increase when BDNF diminished, and also that a reduction in BDNF regulation increased sensitivity to oxidative damage under stressful circumstances.[28] Additionally, in the same study endogenous defense mechanisms against oxygen free radicals were less effective at suppressing stress-induced oxidative damage in the BDNF efficient group. Taken together, these findings suggest that the neuroprotective effect of BDNF can, in particular, promote the suppression of oxidative impairment.

A growing body of evidence indicates that patients with psychiatric disturbances have altered peripheral BDNF levels, especially those with schizophrenia, depression, and PTSD,[29-31] which was not visible in our findings. As for psychiatric comorbidities, only substance abuse showed a positive correlation in our sample. Conversely, a previous study conducted

with a sample of 230 soldiers who participated in combat teams in Iraq and Afghanistan observed that those who developed traumatic stress (n = 41) had higher scores for depression (d = 1.91), anxiety (d = 1.61), poor sleep quality (d = 0.92), post-concussion symptoms (d = 2.21), alcohol use (d = 0.63), traumatic life events (d = 0.42), and exposure to combat (d = 0.91) when compared to those who did not develop traumatic stress.[22]

In the present study, women with PTSD and comorbid depression were 1.52 times more likely to have low BDNF serum concentration (< 25.38 ng/ml) than women without these disturbances (odds ratio [OR] = 1.52; 95% confidence interval [95%CI] 1.00-2.31). Furthermore, women with isolated depression were 1.83 times more likely to have low BDNF serum concentration, albeit not at a non-significant difference (OR = 1.83; 95%CI 0.96-3.46). No association was observed between isolated PTSD and reduced BDNF serum levels (OR = 1.20; 95%CI 0.78-1.87).

A case-control study and metanalysis has explored the role of BDNF Val66Met in susceptibility for PTSD among 265 men across veteran hospitals and the Returned and Services League of Australia. Of the total, 158 participants were diagnosed with PTSD, while 107 had also been exposed to trauma but did not develop this comorbidity. The final cohort totaled 257 participants (PTSD = 151, no PTSD = 106) and underwent chi-square analysis.[32] Based on earlier studies involving both animal models and human participants, the hypothesis formulated in the current study was that individuals who carry the Met allele would be at greater risk for PTSD than those carrying the Val allele.[33]

One study aimed at assessing the association of BDNF levels with PTSD and depression examined a cohort of 2,928 pregnant women (gestational age > 16 weeks) who received treatment at a pre-natal care clinic in Lima, Peru, and found a decrease in BDNF levels in expectant mothers who presented depression and PTSD, but not in those who has PTSD alone.[34] Also, no significant differences were observed in BDNF levels in early maternal pregnancy regarding prepartum PTSD (mean of 21.12 vs. 20.67 ng/ml, p = 0.21). Mean BDNF serum concentrations were lower among women with comorbid PTSD and depression than in those with none of the comorbidities (mean [interquartile range]: 20.44 [16.97, 24.30] vs. 21.35 [17.22, 26.01] ng/ml, p = 0.06), even though the difference was not statistically significant.[34]

In the present study, the following factors were correlated with BDNF levels in 117 patients who were victims of severe physical trauma: collection shift, sociodemographic factors, ASD subtypes, and mental disorders. Seven patients from the sample were also diagnosed with ASD. The group whose blood collection occurred in the afternoon presented significantly greater BDNF values compared to the morning collection. This same group presented lower levels of BDNF when associated with ASD subtypes A, A1, and A2. Regarding mental disorders, only substance abuse was significant in relation to BDNF. Finally, Spearman's correlation showed that rationalization and reactive formation may be associated directly with BDNF levels. It is possible that the low levels of BDNF observed in the morning shift derive from a response to the circadian cortisol cycle, whose action inhibits the expression of serum neurotrophins.

Conclusion

The levels of BDNF in this study were not sufficiently different to distinguish between patients with ASD (only with its subtypes) and without ASD. We consider this a limitation of the study, as only seven patients met ASD criteria. More studies are necessary to obtain a more precise association of BDNF levels with the development of ASD. Perhaps, when this association is clarified, it will be possible to validate the use of this test in clinical practice for posttraumatic stress.

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Disclosure

No conflicts of interest declared concerning the publication of this article.

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